

## Fiber optic biosensing platform for real-time multiplex quantification of bacterial DNA

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Point-of-care (POC) diagnostic tests in microbiology are expected to deliver fast and sensitive detection of bacterial targets. Nowadays, however, detection of molecular targets alone, without their quantification and/or identification of specific mutations is often insufficient<sup>1,2</sup>. Although, different assays have been developed to meet these requirements, including quantitative PCR (qPCR)<sup>3</sup> followed by high resolution melting analysis as well as ligation assays<sup>4</sup>, most of them are still largely incompatible with the concept of POC testing due to their cost or complexity.

In this work, we present the fiber optic (FO) SPR sensor as a platform (Fig1A) for multiplex, real-time monitoring of DNA amplification and detection of single nucleotide polymorphisms (SNPs) in a single sample. FO-SPR has been previously established in our group for monitoring the DNA melting profile by implementing DNA functionalized gold nanoparticles (Au NP) as labels (Fig1B), which allowed discriminating SNPs at high resolution<sup>5,6</sup>. However, to realize simultaneous quantification and cycle-to-cycle identification of the reaction products, the FO-SPR melting assay was combined either with the PCR or with ligation chain reaction (LCR).

The FO-SPR LCR assay amplifies DNA in exponential manner through multiple ligation cycles that progress through 3 succeeding phases: hybridization, ligation and melting phase (Fig1C). The detection limit of the FO-SPR melting assay was drastically improved using the LCR, whereas capacity for SNP detection was preserved (Fig1D). Furthermore, to achieve detection of multiple pathogens within the same sample, FO-SPR melting assay was combined with solution phase PCR using two sets of hybridization probes, one for *Mycobacterium bovis* and the other for *Mycobacterium avium* subsp. *Paratuberculosis*. These two bacteria, which are frequently encountered in life stock, were used in a proof-of-concept study that showed the capacity of FO-SPR melting assay for multiplex DNA detection (Fig1E), thereby further emphasizing the potential of this platform in POC test development.

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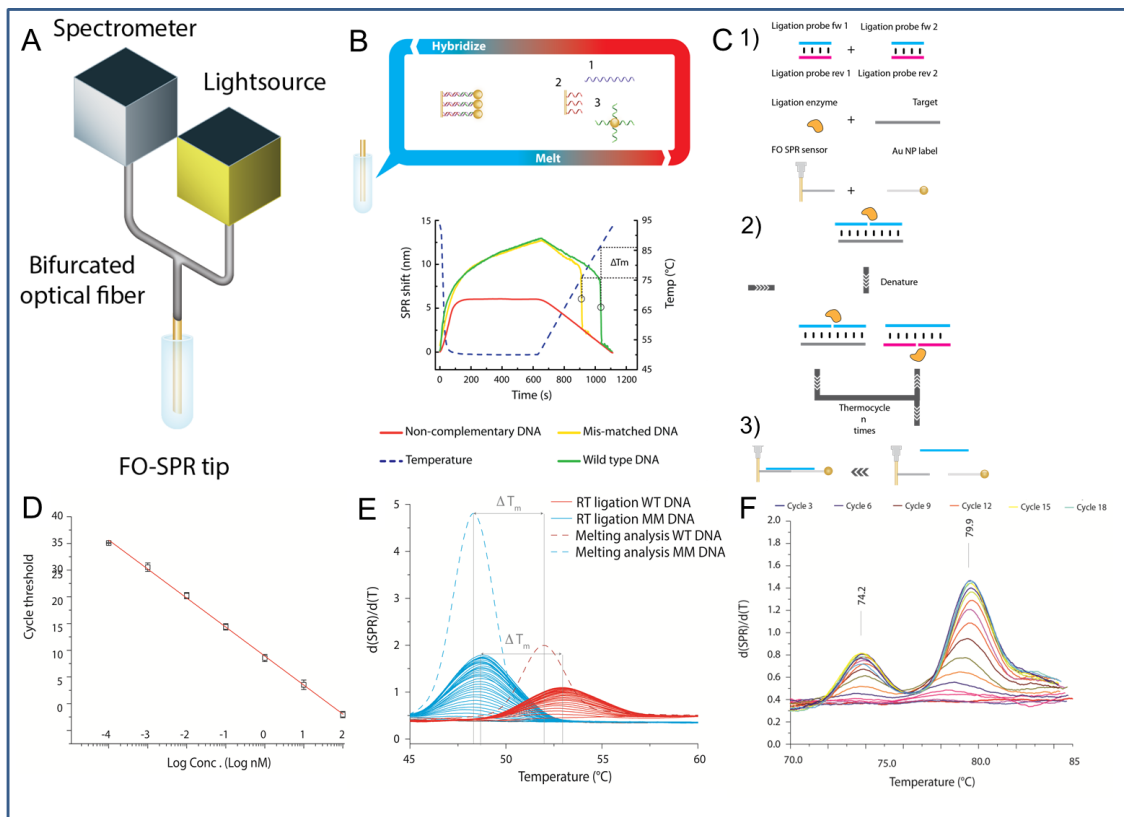


Figure 1. (A) Schematic representation of the FO-SPR setup with all components. (B) Schematic representation of a FO-SPR melting assay (top panel) with DNA target (1) and DNA probes immobilized on the FO-SPR sensor (2) and on Au NP (3). Gene probes hybridize at the FO surface, and are subsequently melted off by a gradual increase of the temperature, resulting in the FO-SPR sensorgram (bottom panel). (C) Schematic overview of the FO-SPR LCR: (1) Different components of the reaction. (2) LCR reaction where the forward and reverse probes are ligated only in the presence of the target sequence, resulting in an exponential amplification during multiple cycles. (3) The forward LCR product can, during the LCR reaction, form a complex with two complementary probes immobilized on the FO-SPR sensor and on Au NPs, allowing real-time monitoring of the reaction. (D) The derived calibration curve with Ct values from the FO-SPR LCR, spans 7 orders of magnitude for DNA concentrations. (E) Obtained signals for WT and MM target DNA using FO-SPR LCR assay (right panel). (F) FO-SPR multiplex PCR, which allows resolving the melting point of the two targets.